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Free-to-Total Prostate-Specific Antigen Ratios 18–24 Months Following External Beam Radiation for Adenocarcinoma of the Prostate

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Background and Objectives: The purpose of this study was to evaluate free-to-total prostate-specific antigen (PSA) ratios after definitive external beam radiation therapy for men with adenocarcinoma of the prostate (CaP).
Methods: A prospective evaluation of percent free PSA in men following definitive external beam radiation therapy for CaP was compared to men with untreated CaP and men at very low risk for CaP. Statistical comparison of clinical and pathologic parameters was performed.
Results: There was no statistically significant difference in free-to-total PSA ratios for men with newly diagnosed CaP and men with detectable PSA who were treated with external beam radiation therapy.
Conclusions: Free-to-total PSA ratios after definitive external beam radiation therapy for CaP are consistent with percent free PSA in patients with newly diagnosed CaP. This supports the theory that PSA from in situ prostate tissue following external beam radiation therapy is produced by malignant cells. *J. Surg. Oncol. 1999;70:91–94.* Published 1999 Wiley-Liss, Inc.†

KEY WORDS: **prostate-specific antigen; percent free PSA; radiotherapy; prostatic neoplasms**

INTRODUCTION

Prostate-specific antigen (PSA), a chymotrypsin-like enzyme directed at liquefaction of the seminal coagulum, is believed to leak from the prostatic ductal system into the prostatic stroma and then into the bloodstream via capillaries and lymphatics. Unlike traditional tumor markers, PSA is not found in larger amounts in tumor cells compared with healthy tissue. In fact, the opposite is true: malignant prostate tissue actually produces less PSA and PSA mRNA than normal prostate epithelial cells and benign prostatic adenomatous tissue [1–3]. The serum concentration of PSA is usually below 2.5 ng/ml, far less than the 5.0 mg/ml found in the seminal fluid.

Original work from Lilja (Malmo, Sweden) [4] and Stenman et al. (Helsinki, Finland) [5] in 1991 demonstrated that the PSA molecule could be measured to exist in different molecular forms in serum. Monoclonal antibodies (Mabs) can be generated against the PSA molecule which recognize one or more of five nonoverlapping conformational immunoreactive epitope groups on the PSA molecule. Current laboratory measurements of free PSA rely on a single epitope remaining accessible to binding by a specific Mab.

Serum PSA activity, at least in vitro, is regulated mainly by two protease inhibitors—alpha-1-antichymotrypsin (ACT) and alpha-2-macroglobulin (AMG)—both

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present in the serum in amounts more than 100 times that of PSA. We believe this same regulation occurs in vivo, although no studies have been performed observing the changing levels of ACT and AMG in vivo and the effect such changes have on PSA activity in vivo. These macromolecules possess the ability to configure covalent complexes with the active form of PSA. After complexing with AMG, no PSA epitopes remain exposed. However, several antigenic epitopes remain exposed after the formation of the PSA-ACT complex, making possible an interaction with a series of anti-PSA Mabs.

Christensson and colleagues [6,7] were the first investigators to determine that the free-to-total PSA proportion (percent free PSA) was significantly lower in patients with prostate cancer (CaP) than in men with benign prostates (0.18 and 0.28, respectively), even at PSA levels lower than 10 ng/ml [1]. Multiple investigators have now confirmed that the application of this parameter results in an increase in specificity of 55–73% for the detection of prostate cancer without compromising the sensitivity of the PSA test for distinguishing prostate cancer from benign prostate tissue [1,8,9].

The reason why patients with prostate cancer display an increased proportion of serum PSA complexed to ACT has not been clarified. It does not appear that the low percentage of free PSA in the serum of these patients is dependent upon the ACT concentration in the serum since ACT is normally present in the serum at much higher molar excess than free PSA. Neither the PSA-ACT concentration nor the percentage of free PSA correlates with the total ACT concentration in the serum [7,10].

It is not known whether the interaction between the enzymatically active PSA and ACT takes place at a pericellular level or in the general circulation. In vitro testing indicates that there is a very low rate of free PSA binding to free ACT. This indirectly indicates that this reaction may occur predominantly at a pericellular level before PSA enters the bloodstream [10]. Immunocytochemistry and in situ hybridization techniques have demonstrated that local PSA-producing prostatic epithelium can synthesize ACT. ACT transcripts and expressed proteins can be detected in most prostate cancer cells [11,12].

While the determination of the clinical utility of free and total PSA in the detection of prostate cancer continues, the effects of different treatment modalities on percent free PSA have not been studied.

Many post-external beam radiation nadir levels of total PSA best predicting subsequent freedom from prostate cancer have been proposed. Although lower nadirs generally are associated with superior outcomes, the identification of a single absolute nadir level was not selected at a recent ASTRO consensus conference. What proportion of post-radiation therapy (XRT) PSA represents bound vs. unbound PSA has not been previously studied. The role free-to-total PSA may play in predicting treatment outcome following XRT for CaP has also not been determined.

MATERIALS AND METHODS

A total of 42 men were prospectively studied with patient informed consent under a protocol approved by the Institutional Review Board.

Peripheral venous sampling of 8–10 ml blood into serum separating tubes (Becton-Dickinson, San Jose, CA) was performed. All samples were allowed to coagulate for 15 min at room temperature, centrifuged at low speed for 10 min, and then immediately frozen at −70°C. Overnight courier was used to ship the frozen samples on dry ice to UroCor, Inc. (Oklahoma City, OK), for analysis.

Assay system consisted of an Immulite solid-phase chemiluminescent detection system (Diagnostic Products Corp., Los Angeles, CA) to quantitate free PSA. Total PSA measurements were quantitated by the commercially applied Tosoh AIA-600 system (Tokyo, Japan), which utilizes an enzyme-conjugated Mab and fluorogenic substrate.

Group 1 consisted of all patients who at the time of the study were 18–24 months from definitive external beam XRT for adenocarcinoma of the prostate, regardless of their Gleason grade or clinical stage at the initiation of treatment (16 men total). All men had received four-field treatment of the prostate and periprostatic tissue. The dose was prescribed at the 100% isodense line of a computer-generated treatment plan. A mean dose of 6,800 cGy was delivered over 48 days in 37 fractions.

Group 2 consisted of 13 men with biopsy-proven adenocarcinoma of the prostate. All men were at least 6 weeks from prostate biopsy and had not received therapy for their CaP prior to venous sampling.

Group 3 consisted of 13 men less than 40 years old, considered to be at low risk for CaP, and without a family history of CaP.

Statistical analysis was performed using a statistical software program (GB-Stat 6.5, Dynamic Microsystems, Inc., Silver Spring, MD). The two-tailed Student *t*-test assuming equal variance (alpha = 0.05) was used to determine statistical significance.

RESULTS

No statistically significant difference existed for clinical tumor stage ($P = 0.696$) or Gleason grade ($P = 0.353$) between men with newly diagnosed CaP and those treated with XRT.

Total PSA in patients with newly diagnosed CaP was higher than the control group ($P = 0.00007$).

Total PSA in patients with newly diagnosed CaP was also higher when compared to total PSA in the post-XRT patients ($P = 0.034$).

Percent free PSA in patients with newly diagnosed

TABLE I. Percent Free PSA in Prostate Cancer Patients Treated With Radiation Therapy

A. Total Vs. Percent Free PSA Results

	Mean total PSA (ng/ml)	Mean percent free PSA
Control	0.5 (±0.23)[a]	49 (±0.29)[a]
Newly diagnosed prostate cancer	11.8 (±8.6)	14 (±0.06)
Prostate cancer treated with XRT	3.2 (±5.8)	16 (±0.11)

B. Statistical Differences in Percent Free PSA

	Control (P)	Newly diagnosed (P)
Control		$P = 0.0003$
Newly diagnosed prostate cancer	$P = 0.0003$	
Prostate cancer treated with XRT	$P = 0.0004$	$P = 0.584$

[a]Standard deviation in parentheses.

CaP was statistically significantly different from the control group, consistent with previous investigators' reports [9,10] ($P = 0.0003$) (Table I).

Percent free PSA in patients 18–24 months post-XRT was (1) significantly lower than the percent free PSA in the control group despite their total PSA not being significantly different ($P = 0.0004$); and (2) not statistically different from the percent free PSA in patients with newly diagnosed prostate cancer ($P = 0.584$).

DISCUSSION

Because the prostate remains in situ after radiation therapy, a logical question has been frequently asked during the 1990s: What level of PSA after XRT could be expected from the in situ prostate and presumably benign glands?

Grob et al. [13] in 1994 performed immune peroxidase histochemistry for expression of PSA on biopsy specimens obtained 12 months after radiation therapy. Results of this study showed that the benign glands identified in these biopsy specimens did not stain the PSA. Only glands that were histologically identified as malignant showed PSA staining. These findings supported the conclusion that serum PSA levels after external-beam radiation therapy were more likely secondary to persistent cancer rather than residual benign prostatic hypertrophy.

Our current study has found that following definitive XRT, free-to-total PSA ratios are consistent with the levels expressed by men with newly diagnosed prostate cancer, and not with levels expressed by men with benign prostatic hypertrophy. This supports the work of Grob et al. [3] that only malignant prostatic epithelial cells express PSA after XRT.

The long-term significance of this finding is unknown. One cannot take these results to indicate a failure of external beam radiation therapy to treat prostate cancer. Should a low free-to-total PSA ratio following XRT represent the persistence of malignant prostatic epithelial cells as concluded in this study, this may not represent clinical failure in the management of the patient's prostate cancer. What critical volume of prostate cancer cells is needed for the disease to be clinically significant is unknown either pre-treatment or post-treatment.

Clinical application of the newly available free-to-total PSA assay to patients treated with external beam radiation therapy is not yet defined. While Pearson et al. [14] have been able to retrospectively predict CaP in men 8 years in advance of the diagnosis using percent free PSA measurements of stored serum samples, this predictive value of percent free PSA has not been demonstrated in patients who have received definitive external beam therapy for their disease.

CONCLUSIONS

Percent free PSA (free-to-total PSA ratio) in men 18–24 months after definitive external beam radiation therapy for CaP is consistent with percent free PSA in patients with newly diagnosed adenocarcinoma of the prostate. This supports the theory that PSA from in situ prostate tissue following external beam radiation therapy for adenocarcinoma of the prostate is produced by malignant cells. The clinical implication of this observation is unknown.

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REFERENCES

1. Chu TM: Prostate-specific antigen. In Sell S (ed): ''Serological Cancer Markers.'' Totowa, NJ: Humana Press, 1992:199–215.
2. Papsidero LD, Kuriyama M, Wang MC, et al.: Prostate antigen: A marker for human prostate epithelial cells. J Natl Cancer Inst 1981;66:37–42.
3. Qiu SD, Young CY, Billhartz DL, et al.: In situ hybridization of prostate-specific antigen mRNA in human prostate. J Urol 1990;144:1550–1556.
4. Lilja H, Christensson A, Dahlén U, et al.: Prostate-specific antigen in serum occurs predominantly in complex with α-1-antichymotrypsin. Clin Chem 1991;37:1618–1625.
5. Stenman UH, Leinonen J, Alfthan H, et al.: A complex between prostate specific antigen and alpha-1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: Assay of the complex improves clinical sensitivity for cancer. Cancer Res 1991;51:222–226.
6. Christensson A, Lilja H: Complex formation between protein C inhibitor and prostate-specific antigen in vitro and in human semen. Eur J Biochem 1994;220:45–53.
7. Christensson A, Björk T, Nilsson O, et al.: Serum prostate-specific antigen complexed to alpha-1-antichymotrypsin as an indicator of prostate cancer. J Urol 1993;150:100–105.
8. Catalona WJ, Smith DS, Wolfert RL, et al.: Evaluation of percentage of free serum prostate-specific antigen to improve specificity of prostate cancer screening. JAMA 1995;274:1214–1216.
9. Oesterling JE, Wojno KJ, England B, et al.: A comparison of free

to total PSA (F/T) ratio to total PSA for distinguishing benign prostatic hyperplasia (BPH) from prostate cancer (CaP) using the Abbott AxSym system. J Urol 1996;155:370A.
10. Abrahamsson PA, Lilja H, Oesterling JE: Molecular forms of serum PSA: The clinical value of percent free-PSA. Urol Clin North Am 1997;24:353–366.
11. Bjartell A, Björk T, Matikainen MT, et al.: Production of alpha-1-antichymotrypsin by PSA-containing cells of the human prostate epithelium. Urology 1993;42:502–510.
12. Björk T, Bjartell A, Abrahamsson PA, et al.: Alpha-1-antichymotrypsin production in PSA-producing cells is common in prostate cancer but rare in benign hyperplasia. Urology 1994; 43:427–434.
13. Grob BM, Schellhammer PF, Brassil DN, et al.: Changes in immunohistochemical staining of PSA, PAP and TURP-27 following irradiation therapy for clinically localized prostate cancer. Urology 1994;44:525–529.
14. Pearson JD, Luderer AA, Metter EJ, et al.: Longitudinal analysis of serial measurements of free and total PSA among men with and without prostatic cancer. Urology 1996;48:4–9.